

Evaluation of the Local Irritation Potential of Hypertonic Saline–Dextran (HSD) in Mice and Rabbits

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Recent questions have renewed concerns regarding possible irritation associated with intravenous (i.v.) injection of 7.5% hypertonic saline (HS) or hypertonic saline–dextran (HSD: 7.5% NaCl and 6% Dextran-70). This study investigated local injection site irritation associated with i.v., paravenous (p.v.), intramuscular (i.m.) and subcutaneous (s.c.) injection of HSD or its individual components. Mice ($n = 10$ per group per time point) and rabbits ($n = 10$ per group per time point) were infused i.v. with the maximum tolerated dose (28 or 20 ml kg⁻¹, respectively) of HSD, HS, Dextran-70 (D-70) or lactated Ringer's solution (LR). Animals were observed at 1, 2 and 4 h after injection and then twice daily until euthanized on day 3 or 14. In irritation studies, 24 rabbits were randomized to receive the four fluids and they were evaluated histologically at 4, 24, 48 or 72 h after i.v., p.v., i.m. or s.c. infusion. The sites were observed immediately after injection, at 4 h and then twice daily until euthanasia. In surviving mice, bruising of the tail was observed in 6/18 and 5/19 animals in the HSD and HS groups, respectively, compared with 0/20 animals in the D-70 or LR groups. Sloughing of the tail was eventually observed in two HSD-infused and three HS-infused mice, compared with none in the other groups. More bruises, hematomas and blebs were observed after i.v. or s.c. injection of HS and D-70 than LR or HSD in the rabbit irritation studies, but the differences among groups were not statistically significant. In the acute toxicity study in rabbits, bruising at the site of injection was observed in 7/20 and 5/14 surviving animals from the HSD and HS groups, respectively, but none was observed in the LR or D-70 groups. These data suggest that, if infused over 5–10 min into a peripheral or central vein, a therapeutic dose of HSD (4 ml kg⁻¹) should not induce any greater inflammation on the vein than LR. However, if significant extravasation of hypertonic fluid occurs, the possibility of localized, focal necrosis might be expected to occur. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

Over the past 15 years, a number of preclinical and clinical studies have supported the use of 7.5% NaCl 6% Dextran-70 (HSD) for the treatment of hemorrhagic hypotension (Smith *et al.*, 1985; Hannon *et al.*, 1989, 1990; Wade *et al.*, 1989; Vassar *et al.*, 1990, 1991; Mattox *et al.*, 1991). As part of this evaluation, studies have also addressed potential adverse effects and toxicity associated with HSD (Vassar *et al.*, 1990, 1991; Dubick *et al.*, 1991, 1993; Mattox *et al.*, 1991; Summary *et al.*, 1992). Recently, an Institute of Medicine report recommended the use of 7.5% hypertonic saline for the treatment of hemorrhagic hypotension (Pope *et al.*, 1999), but concerns have remained regarding the potential for local toxicity associated with the infusion of hypertonic fluids, such as the severity of irritation or tissue

injury at the injection site. This paper summarizes three separate studies with HSD and its individual components that evaluated local irritation and toxicity related to the infusion of these compounds and compares the results to an equal volume of lactated Ringer's solution (LR). These studies were part of the overall pre-clinical toxicology studies to support the new drug application for HSD filed with the US Food and Drug Administration (FDA).

MATERIALS AND METHODS

These studies were approved by the Animal Care and Use Committee of the former Letterman Army Institute of Research, Presidio of San Francisco, CA, where the work was performed. They complied with FDA guidelines and institutional policy for conducting acute intravenous toxicity and irritation studies. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and by the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

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Studies in mice

Male ($n = 40$) and female ($n = 40$) ICR mice weighing 16.5–26.9 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). Animals were housed individually in stainless-steel cages with screen bottoms in rooms controlled for temperature, light and humidity, and were fed Purina rodent chow and water *ad libitum*. Mice were randomized to receive the four fluids mentioned below ($n = 5$ males and 5 females per fluid group per time point) using a computer-based, stratified, weight-biased method (XYBION Path/Tox AESLCT Animal Allocation Program). All animals were infused over 5 min with the previously estimated maximum tolerated dose of 28 ml kg^{-1} of 7.5% hypertonic saline 6% Dextran-70 (HSD) or its individual components, 7.5% NaCl (HS) or Dextran-70 (D-70), via the tail vein. Injections were made using a tuberculin syringe and 26-gauge, $\frac{3}{4}$ -inch stainless-steel needles. Comparable volumes of lactated Ringer's solution (LR) were infused as the control fluid. Mice were observed at 1, 2 and 4 h on the day of injection and twice daily thereafter until euthanized for necropsy on day 3 or 14 after injection.

Studies in rabbits

Male New Zealand White rabbits ($n = 24$) initially weighing 2.0–2.6 kg were obtained from Elkhorn Rabbitry (Watsonville, CA) and housed in stainless-steel cages with screen bottoms in a room controlled for temperature, light and humidity. Rabbits were fed Purina rabbit chow and water *ad libitum*. These rabbits for the local irritation studies were randomized for testing of the four fluids (HSD, HS, D-70 and LR). Each rabbit was tested with two of the four fluids (randomized to the left or right ear or left or right side of the body) and evaluated grossly and histologically at 4, 24, 48 and 72 h after fluid administration (Table 1) in accordance with FDA guidelines for conducting a local irritation bioassay (FDA, 1978). Infusions were made into four sites: intravenous (i.v.), paravenous (p.v.), intramuscular (i.m.) and subcutaneous (s.c.). A dose of 0.5 ml at a rate of 0.1 ml in 10 s was given i.v. through the marginal ear vein, the p.v. dose was 0.1 ml in 10 s infused immediately medial to the marginal ear vein, the i.m. dose was 0.5 ml in 10 s infused into the lateral compartment of the thigh and the s.c. dose was 0.5 ml in 10 s into the dorsolateral thoracic region immediately caudal to the scapula. All infusions used tuberculin syringes with 23-gauge, $\frac{3}{4}$ -inch stainless-steel needles. The area around each injection site was marked with ink.

In a separate study evaluating the acute toxicity of intravenous HSD and its individual constituents, male ($n = 40$) and female ($n = 40$) New Zealand White rabbits (2.4–3.7 kg) were obtained from Hazleton Research Products, Inc (Denver, PA). Rabbits were randomized ($n = 5$ males and 5 females per group per time point) to receive the four fluids (HS, HSD, D-70 or LR) i.v. via the marginal ear vein, at the previously estimated maximum tolerated dose of 20 ml kg^{-1} over a 5-min period. Infusions were made using a 60-cc syringe and 23-gauge 1-inch stainless-steel needles. Other details of rabbit husbandry were as described above. Rabbits were observed at 1, 2 and 4 h on the day of injection and twice daily thereafter until euthanized for necropsy on day 3 or 14 after injection.

For histological assessment, skin samples, the marginal ear vein and skeletal muscle were fixed in 10% neutral buffered formalin, embedded in paraffin and sections stained with hematoxylin and eosin. Sections of skin were taken from the actual site of injection and 1 cm distal and 1 cm proximal to the injection site. Skeletal muscle samples were taken from injection sites of the thigh that exhibited gross changes. All tissue was evaluated by routine light microscopy.

Statistical analysis

Clinical observations of injection site irritation or toxicity were tabulated by groups. Data were analyzed by Fisher's Exact Test; $P < 0.05$ was considered statistically significant after Bonferroni correction for multiple non-orthogonal comparisons.

RESULTS

Observations in mice

There were two deaths in the HSD and one in the HS group within 12 min of the start of infusion of the maximum tolerated dose (28 ml kg^{-1}). No other mortality was observed in any group during the 14-day study. As a consequence, only 18 and 19 mice were available for evaluation in the HSD and HS groups, respectively.

Bruising at the injection site of the tail was observed in 6/18 surviving mice in the HSD group and in 5/19 mice in the HS group. Pathological evaluation of the bruising in HSD-infused mice was considered very slight in two, moderate in three and very severe in one mouse. Bruising in HS-infused mice was very slight in two, moderate in two and very severe in one mouse. Eventual tail sloughing distal to the injection site occurred in two HSD-infused and three HS-infused mice. None of these effects were observed in mice infused with 28 ml/kg D-70 or LR nor were any gender-specific effects of HSD or HS infusion noted.

Observations in rabbits

In the acute toxicity studies there were six deaths (3 male and 3 female) after i.v. infusion of 20 ml kg^{-1} HS in rabbits. These deaths occurred early, within 3–12 min after the start of infusion. There was no other mortality in any group during the study period. Consequently, there were only 14 rabbits in the HS group for evaluation in the acute toxicity study.

Infusion of 20 ml kg^{-1} of the test fluid resulted in bruising at the injection site in 7/20 rabbits in the HSD group and in 5/14 surviving animals in the HS group. In addition, fluid or bloody discharge, discoloration or necrosis at the injection site was observed in 3/20 and 5/14 rabbits in the HSD and HS groups, respectively. Bruising or necrosis at the injection site was not observed in rabbits infused with LR or D-70 but bloody discharge or discoloration was observed in 2/20 and 1/20 rabbits in these groups, respectively. No gender-related differences in frequency of these observations were seen in any group. In addition, no gross or histological evidence of damage to the rabbit marginal ear vein itself was noted in response to infusion of any of the fluids.

Table 1 Injection site clinical observations in rabbits^a

Time (h)	HSD						LR						HS						DEX-70					
	H	B	L	N	E	<i>n</i>	H	B	L	N	E	<i>n</i>	H	B	L	N	E	<i>n</i>	H	B	L	N	E	<i>n</i>
Intravenous																								
0	–	–	–	–	–	11	–	–	–	–	–	11	1	–	–	–	–	12	–	–	–	–	–	12
4	–	–	–	–	–	11	–	–	–	–	–	11	1	3	–	–	–	12	2	1	–	–	–	12
24	–	1	–	–	–	9	–	1	–	–	–	9	–	5	–	–	–	9	–	4	–	–	–	9
48	–	1	–	–	–	6	–	3	–	–	–	6	–	3	–	–	–	6	–	3	–	–	–	6
72	–	1	–	–	–	3	–	–	–	–	–	3	–	1	–	–	–	3	–	–	–	–	–	3
Total	–	3	–	–	–	11	–	3	–	–	–	11	1	7	–	–	–	12	2	5	–	–	–	12
Paravenous																								
0	–	–	1	–	–	11	–	–	1	–	–	11	–	–	2	–	–	12	–	–	2	–	–	12
4	–	1	–	–	–	11	–	–	–	–	–	11	–	1	–	–	–	12	–	–	–	–	–	12
24	–	1	–	–	–	9	–	–	–	–	–	9	–	1	–	–	–	9	–	1	–	–	–	9
48	–	1	–	–	–	6	–	2	–	–	–	6	–	2	–	–	–	6	–	–	–	–	–	6
72	–	1	–	–	–	3	–	–	–	–	–	3	–	1	–	–	–	3	–	–	–	–	–	3
Total	–	1	1	–	–	11	–	–	1	–	–	11	–	2	2	–	–	12	–	1	2	–	–	12
Intramuscular																								
0	–	–	–	–	–	11	–	–	–	–	–	11	–	–	–	–	–	12	–	–	–	–	–	12
4	–	–	–	–	–	11	–	–	–	–	–	11	–	–	–	–	–	12	–	–	–	–	–	12
24	–	–	–	–	–	9	–	–	–	–	–	9	–	–	–	–	–	9	–	–	–	–	–	9
48	–	–	–	–	–	6	–	–	–	–	–	6	–	–	–	–	–	6	–	–	–	–	–	6
72	–	–	–	–	–	3	–	–	–	–	–	3	–	–	–	–	–	3	–	–	–	–	–	3
Total	–	–	–	–	–	11	–	–	–	–	–	11	–	–	–	–	–	12	–	–	–	–	–	12
Subcutaneous																								
0	–	–	2	–	–	11	–	–	2	–	–	11	–	–	5	–	–	12	–	–	5	–	–	12
4	–	–	1	–	–	11	–	–	–	–	–	11	–	–	1	–	–	12	–	–	–	–	–	12
24	–	–	–	–	–	9	–	–	–	–	–	9	–	–	–	–	–	9	–	–	–	–	–	9
48	–	–	–	–	–	6	–	–	–	–	–	6	–	–	–	–	–	6	–	–	–	–	–	6
72	–	–	–	–	–	3	–	–	–	–	–	3	–	–	–	–	–	3	–	–	–	–	–	3
Total	–	–	2	–	–	11	–	–	2	–	–	11	–	–	5	–	–	12	–	–	5	–	–	12

^a Clinical observation key (values indicate incidence of observed sign): H = hematoma; B = bruise; L = bleb; N = nodule; E = erythema; *n* = total number of animals in group; HSD = 7.5% NaCl–6% Dextran-70; LR = lactated Ringer's; HS = 7.5% NaCl; DEX-70 = 6% Dextran-70; Total = total number of injection sites exhibiting the sign at least once during the study period.

In the injection site irritation studies, the incidences over time of bruising, hematomas, blebs, nodules and erythema after infusion of the four test fluids are summarized in Table 1. Groups with $n = 11$ indicate that a rabbit died before dosing began and was not replaced. The highest incidence of bruising was observed in rabbits infused i.v. with HS (7/12) and D-70 (5/12), whereas the incidence of bruising was 3/11 in both the HSD- and LR-infused animals (Table 1). There were no statistically significant differences in the incidence of bruising among groups. In all groups, bruising tended to develop within 24–48 h of injection and decreased in size over time. Hematomas observed in the HS and D-70 groups after i.v. infusion resolved into bruises (Table 1). Importantly, no histological evidence of vein damage was observed after infusion of any fluid.

No significant differences in incidence of bruising were observed among groups after p.v. infusions and no bruising was observed after i.m. or s.c. injections. The incidence of intradermal injection blebs (blisters) was similar among groups after p.v. infusion and blebs tended to resolve within 4 h after injection without residual effects. These blebs formed immediately after s.c. injections and their incidence was highest in the HS and D-70 groups (Table 1). All blebs resolved by 24 h after injection. Nodules and erythema were not observed in any group at any injection site during the experimental period.

The most common histological lesion observed in all four groups associated with the injection site was hemorrhage. Acute dermatitis of the ear was observed in 5/11 rabbits in the LR group, 3/11 in the HSD group, 5/12 in the HS group and 5/12 in the D-70 group. Ulcerative dermatitis was only observed in 1/12 rabbits in the HS group. Inflammation of thigh skeletal muscle was observed in 2/11 rabbits in both the LR and HSD groups, but was not observed in any of the HS- and D-70-infused rabbits. This inflammation was also associated with mild muscle degeneration in 2/11 and 1/11 rabbits in the LR and HSD groups, respectively. This mild muscle degeneration was attributed to introduction of foreign material, such as fur, during needle insertion. These observations were considered likely to resolve with no morphological or functional alterations and therefore were unlikely to be clinically significant.

DISCUSSION

In mice, deaths observed in the HSD group suggest that their maximum tolerated dose is actually less than the 28 ml kg⁻¹ infused. Similarly, in the acute toxicity study in rabbits, deaths in the HS group suggest that 20 ml kg⁻¹ exceeds the maximum tolerated dose for HS in this species. Nevertheless, these doses are still seven or five times, respectively, the recommended therapeutic dose of ca. 4 ml kg⁻¹ (250 ml in a 60–70 kg person) for humans.

Generally, the data from both the present mouse and rabbit acute toxicity studies support the observation that the injection site clinical findings after HSD infusion are consistent with large-volume (≥ 20 ml kg⁻¹) infusions of HS. For example, because extravasation of fluid was an

unavoidable consequence of trying to infuse such large volumes into the mouse tail vein, it is believed that the tail sloughing distal to the infusion site was due to induction of necrosis by a direct effect of the hypertonic saline component of HSD. Such an effect could be expected because intradermal HS injection has been used as a nociceptive test in mice (Hwang and Wilcox, 1986) and is most likely volume (or dose) related because HSD was not observed to be irritating in a standard irritation test system (Zaucha *et al.*, 1988). In addition, in a recent study in which human volunteers were bled 8–10 ml kg⁻¹ and infused with 250 ml (a therapeutic dose) of HSD or its individual components, pain at the injection site was reported in 1/10 and 3/9 patients who received HSD or HS, respectively (L.O. Lindbom, BioPhausia, personal communication). Because soreness at the injection site is a common complaint of many therapeutic trials, the clinical significance of the current observations, short of overt tissue damage, seems minimal.

The results from the injection site evaluation in rabbits suggested that the presence of bruises was simply a consequence of perivascular leakage of blood from the needle insertion, and most bruises resolved by 72 h after injection. In addition, the occurrence of blebs after p.v. and s.c. injections were considered to be a typical reaction to the injection technique. Thus, at multiple injection sites, the dose of HSD or HS was not significantly more irritating than LR. These gross observations were confirmed by lack of significant histological findings of the tissues associated with the different injection sites. These data are consistent with the study by Hands *et al.* (1988), who observed no adverse morphological effects on peripheral or central veins 8 days after three infusions of 5 ml kg⁻¹ of HSD, 4 days apart. In addition, Pascual *et al.* (1993) observed only minimal inflammation in the marginal ear veins of swine infused with 6.3 ± 1.3 ml kg⁻¹ of HSD to a physiological endpoint.

In conclusion, the mouse and rabbit acute toxicity data would suggest exercising caution in the rapid infusion of large volumes (≥ 20 ml kg⁻¹) of HS or HSD into a peripheral vein because the HS component can induce tissue injury and pain at the injection site, particularly if extravasation of fluid occurs. However, the rabbit irritation study suggests that low doses of HS or HSD are not significantly more irritating than LR when infused through various routes. Taken together, these data suggest that, if infused over 5–10 min into a peripheral or central vein, a therapeutic dose of HSD (4 ml kg⁻¹) should induce minimal inflammation. However, if significant extravasation of hypertonic fluid occurs, the possibility of localized, focal necrosis might be expected to occur.

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